

INTERACTIONS OF DIURNAL AND TIDAL RHYTHMS IN THE FIDDLER CRAB, *UCA PUGNAX*¹

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One of the most nearly universal characteristics of circadian rhythms is the susceptibility to phase-shifting by light. It has been shown to be effective not only for rhythms which under natural conditions are synchronized with a solar-day cycle but also for some which are tidal in nature. Where a diurnal and a tidal rhythm are simultaneously present it is of interest to determine the characteristics of both rhythms with respect to their relative susceptibility to shifting by light. A species known to possess both of these rhythms is the fiddler crab, *Uca pugnax*. Rhythms of both solar-day and tidal periods have been described for color change, oxygen consumption and locomotor activity. The susceptibility of the daily rhythm of color change to shifting by light has been extensively investigated and the characteristics are well established (Webb, 1950). It has also been found that a light-induced shift of the diurnal rhythm is accompanied by a shift of the tidal rhythm, the shifts being of approximately the same magnitude (Brown *et al.*, 1953). This situation suggests either a coupling of some kind between the two rhythms or very similar sensitivities and reactions. Since the expression of the two rhythms requires a continuously changing phase relationship between them it is obvious any coupling that may exist is not total.

Less extensive investigations of the characteristics of the rhythms of locomotor activity have been reported. The crabs show peaks of locomotor activity near the times of low tide and these phase relations are established by some factor associated with the local tides. Thus, animals from two beaches in the same general area but with tide times five hours different show tidal rhythms out of phase with each other by about five hours (Bennett, Shriner and Brown, 1957). There is no evidence that, under such circumstances, the diurnal rhythms are out of phase with each other.

The purpose of this report is to describe the tidal rhythm of locomotor activity of *Uca pugnax* under a variety of light regimes, and some of the characteristics of the apparent linkage between the diurnal and tidal rhythms.

EXPERIMENTAL METHODS

The locomotor activity of male *Uca pugnax*, collected at Chappaquoit Beach, Cape Cod, was recorded by the method described by Bennett, Shriner and Brown

¹ This study was aided by grants from the National Institutes of Health (GM 07405) and the National Science Foundation (G-15008) and by a contract with the Office of Naval Research (1228-30).

in 1957. In some cases the animals were placed in the recording apparatus on the day of collection and the recording carried out under conditions of constant light at an intensity of less than 1 f.c. In some cases recording, under the same conditions, was begun on the day after collection. Since there was no discernible difference between results obtained in the two cases the data have been combined. A total of 70 days of data obtained under these recording conditions is available from two different years, 1961 and 1964, representing about 12 different collections. The times of low tide on the days of collection were distributed through the daylight hours.

Activity was also recorded under conditions of natural illumination with the animals protected from direct sunlight by a drawn window shade. Two simultaneous series of records, each for a 12-day period, were obtained during September, 1964. The animals were placed in the activity chambers the day after collection. Three simultaneous series, each for a 28-day period, were recorded from June 28 to July 26, 1962. These animals were exposed to an artificial schedule (LD, 12:12; L 50 f.c.) for five days prior to recording activity which was carried out under LL conditions, (L 1 f.c.). Three types of LD cycles were used: (1) light on 6 AM to 6 PM; (2) light on 2 PM to 2 AM; and (3) light on 10 PM to 10 AM. At the end of 15 days the animals in the activity chambers were replaced by other animals that had been pretreated in the same ways.

A total of 40 days of recordings was obtained in 1964 under conditions of constant light of approximately 1 f.c. but with a supplemental lighting of two hours at 55 f.c. The supplemental lighting provided a 24.75-hour cycle since the light period began 45 minutes later each day.

Finally a series of 11 days of data was obtained in 1964 with conditions of constant light at 1 f.c. and with a 24.75-hour cycle provided by means of a supplemental light of 55 f.c. during four hours of each cycle.

METHODS OF ANALYSIS

Locomotor activity for each hour of each test period was expressed as per cent of hour active, each such value representing the average of from 3 to 6 animals. The time of occurrence of the highest value for each half-day was then recorded. Next, the times of occurrence of local low tides were recorded for each day for which data were available. Finally, the time of highest activity for each half-day was plotted as a function of the time of nearest low tide. When all the data for a given set of conditions are plotted together in the manner described, and similar plots made for each set of conditions one should be able to answer two questions. (1) Is there a characteristic relation between the time of occurrence of maxima and time of low tide? (2) If such a relationship exists is it influenced by pretreatment or conditions of recording? The method obviously places certain arbitrary limits on the time relations possible. But if the high values are distributed randomly with respect to time of day and time of tide one would obtain a plot with points falling randomly within a diagonal band whose limits are time of low tide plus and minus 6 hours. If, on the other hand, there is a consistent relationship to time of tide, only a portion of this band would be occupied. An advantage of this method of plotting is that if there is a systematic variation of the time relationships occurring throughout the lunar month, such a variation should be evident in the graph. The

usual method of obtaining a mean lunar-day curve requires the averaging of values for an unbroken series of data for 15 days or, preferably, for 29 or 30 days, and obviously does not show the nature of any systematic variations within the period for which the curve is obtained.

RESULTS

In Figure 1 are plotted the data for all of the experiments in which there was no pretreatment and recording was under LL conditions. Ordinate values repre-

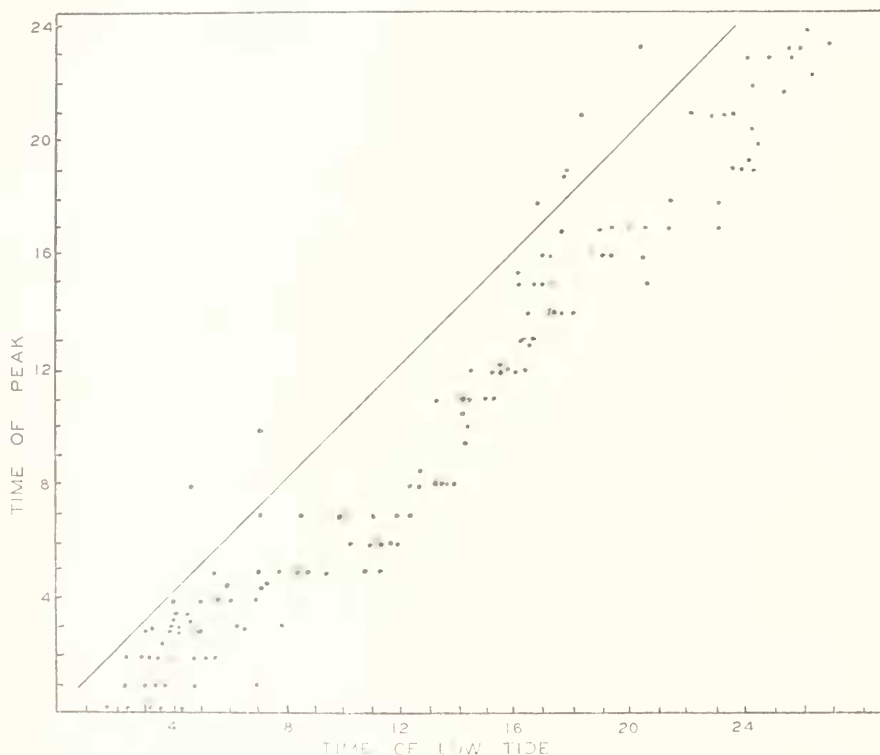


FIGURE 1. Time of day at which peak of locomotor activity occurs (ordinate) in relation to time of day of nearest low tide (abscissa). Recording in constant illumination. The diagonal represents the positions of peaks if they coincided with times of low tides.

sent time of maximum activity, abscissal values are time of occurrence of low tide. The vast majority of points fall between the time of low tide and six hours before low tide. It is evident, however, that between hours 1 and 5 of the solar day many points fall in the three hours before low tide, while between hours 8 and 14 of the solar day practically all of the points lie between 3 and 6 hours before low tide. In other words, while the tides are progressing from about 6 AM to noon, there is almost no corresponding progression in time of occurrence of activity maxima. There then occurs a rapid progression of the activity maxima so that by

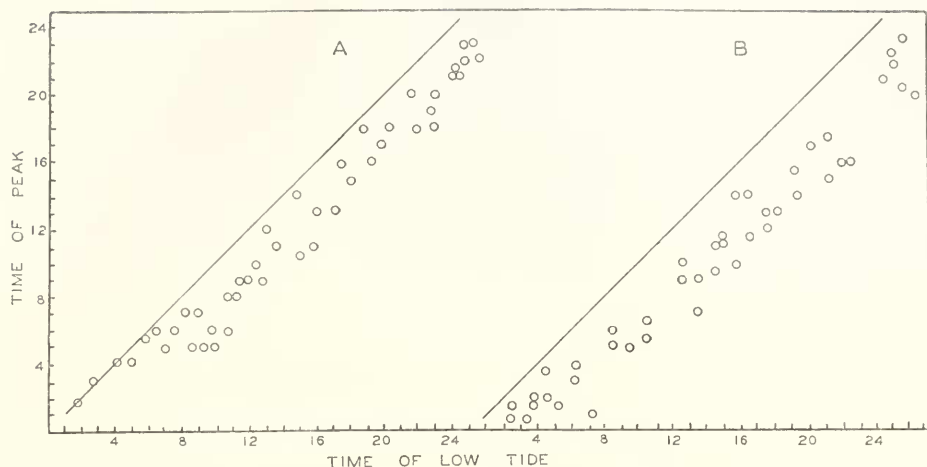


FIGURE 2. A. Time of activity peaks in relation to time of low tides for animals pretreated with light 6 AM to 6 PM and recorded in constant illumination. B. Times of activity peaks for animals without pretreatment and recorded in natural illumination.

the time the tides occur at 17 hours the peaks occur at almost the same time. It should be noted that these variations were not dependent on the length of time the animals had been in the laboratory or in the recorders.

Figures 2A and B show comparable data for animals pretreated by exposure to 5 days of light on from 6 AM to 6 PM (2A) and for animals recorded under conditions of natural illumination (2B). In both cases the majority of points fall in the

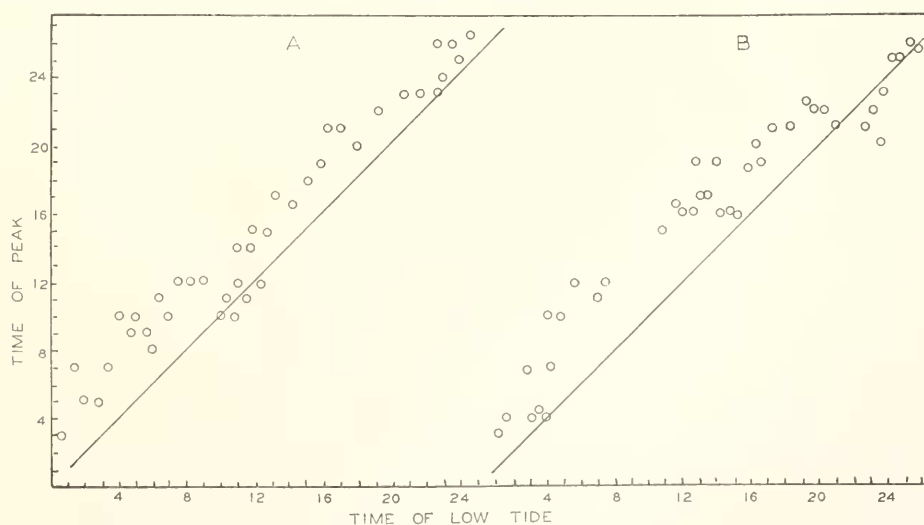


FIGURE 3. A. Time of activity peaks in relation to time of low tide for animals pretreated with light 2 PM to 2 AM and recorded in constant illumination. B. Time of activity peaks for animals pretreated with light 10 PM to 10 AM; recorded in constant illumination.

6 hours preceding low tide. The same tendency of the peaks to remain stationary while the tides progress from 6 AM through the morning hours is seen in Figure 2A as was noted in Figure 1. However, there is no such delay evident in Figure 2B. Thus, the exposure to natural illumination seems to have resulted in a more accurate expression of the tidal rhythm on a day-to-day basis—but not more accurate on the average.

The data obtained from animals pretreated by exposure to 5 days of light from 2 PM to 2 AM are shown in Figure 3A, and those for animals pretreated by exposure to light from 10 PM to 10 AM are seen in Figure 3B. In contrast with any of the preceding cases the majority of these points are seen to fall in the period following

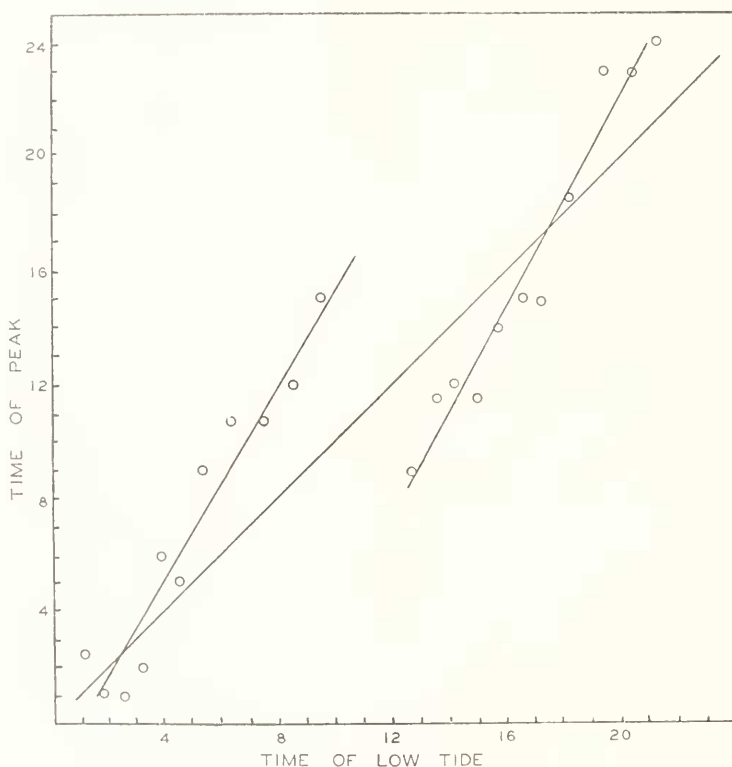


FIGURE 4. Time of activity peaks in relation to low tide for animals exposed during recording to cycle of 24.75 hours by four-hour supplemental light.

the time of low tide. In Figure 3A a delay in progression of the peaks occurs, such that as the time of low tide approaches noon the peaks coincide with it or even precede it; a similar condition seems to occur just before midnight. Similarly in Figure 3B, there is conspicuous delay in progression of the peaks as tides move from about 6 PM to midnight.

The data obtained for animals exposed to a 24.75-hour cycle by supplemental light periods of four hours duration are seen in Figure 4. The points in this

case seem not show any systematic variation in rate of progression but rather to progress consistently at a rate greater than that of the tides. The period calculated from the slope of the line drawn through the points is about 25.5 hours.

The data for animals exposed to a 24.75-hour cycle by supplemental light periods of two hours duration are seen in Figure 5. There was less consistency between samples here than for any of the previous experiments. In a few cases the average rate of progression of peaks over a period of 10 or more days was greater

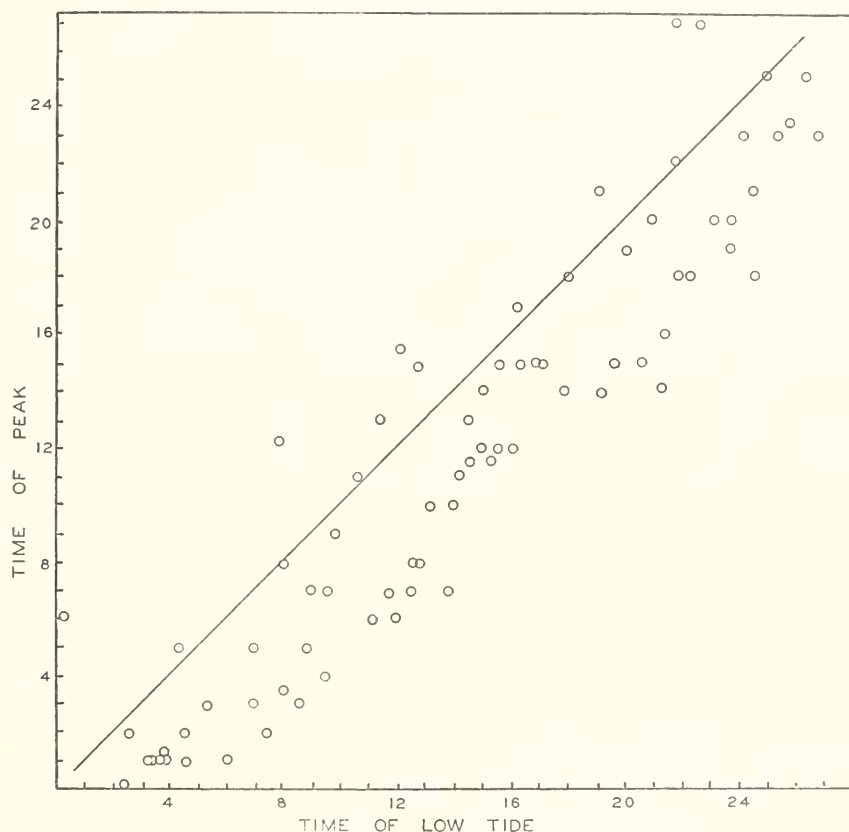


FIGURE 5. Time of activity peaks in relation to low tide for animals exposed during recording to cycle of 24.75 hours by two-hour supplemental light.

than the rate of tidal progression; in most cases the peaks occur within the six-hour period preceding low tide but with no clear pattern within that period.

In Figure 6 are presented mean diurnal curves for those experiments for which two successive 15-day periods of data were available. Figure 6B shows the mean curve for animals pretreated by exposure to light from 6 AM to 6 PM for 5 days; ordinate values are per cent of hour active, along the abscissa are plotted hours of the solar day. Figure 6C presents comparable data for animals pretreated by exposure to light from 10 PM to 10 AM; Figure 6D, the data for animals exposed

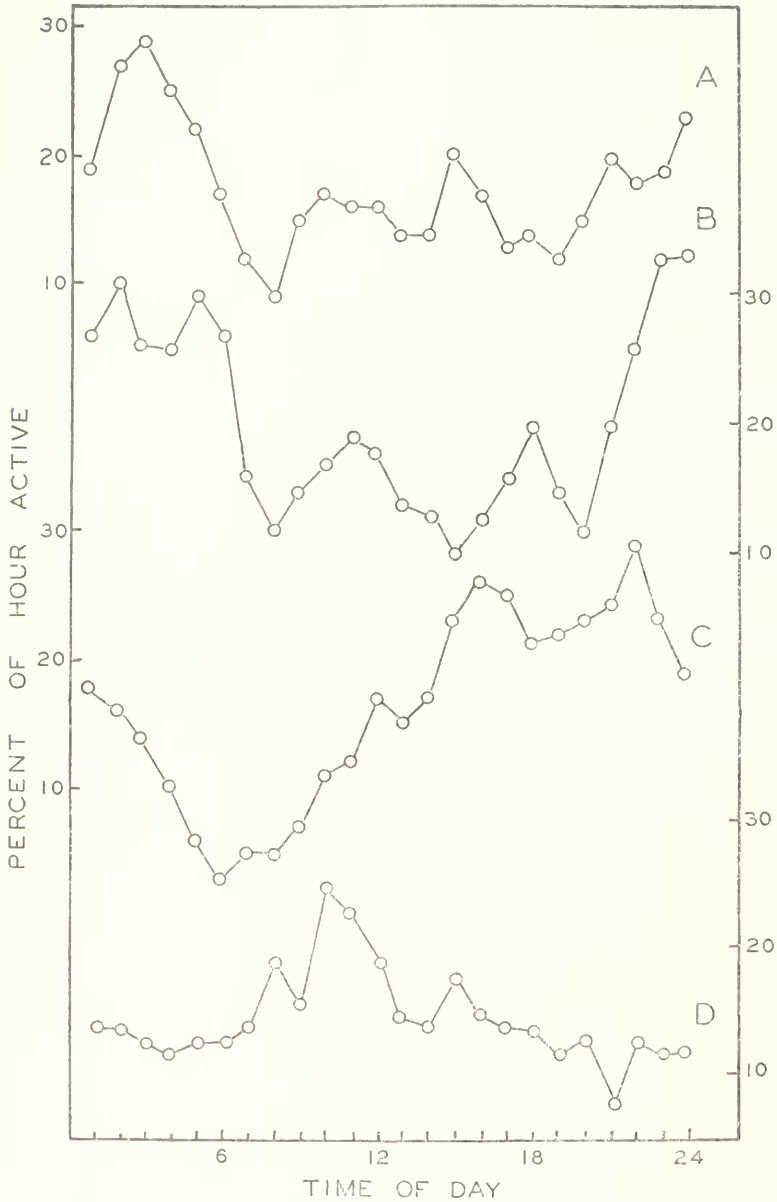


FIGURE 6. Mean diurnal curves: A. For animals without pretreatment. B. For animals pretreated with light 6 AM to 6 PM; C. For animals pretreated with light 10 PM to 10 AM; D. For animals pretreated with light 2 PM to 2 AM. All recorded in constant illumination.

to light from 2 PM to 2 AM; Figure 6A, the data for animals without pretreatment and recording begun within a day of collection. Animals that had experienced an LD cycle essentially in phase with the normal (Fig. 6B) show a high level of activity from about 10 PM to 6 AM. Animals whose artificial dawn was 8 hours

early (-120°) show a high level of activity from about 3 PM to midnight (Fig. 6C). Their cycle is thus shifted by 6 to 7 hours. Animals whose artificial dawn was 8 hours late ($+120^\circ$) show a peak of activity at about 10 AM (Fig. 6D). Using the mid-point of activity seen in Figure 6B as a basis for comparison, one arrives at an estimated shift of 8 hours for the curve in Figure 6D. The phase relations of the diurnal rhythm of the untreated animals (Fig. 6A) seem to be the same as those seen in Figure 6B.

DISCUSSION

The results of these experiments suggest two kinds of relationship between diurnal and tidal rhythms of locomotor activity. One kind of relation is seen for all animals whose activity was recorded under conditions of constant light. Under these conditions systematic variations in position of activity peaks relative to the time of low tide were consistently observed. In these cases the use of position of the peak to estimate the period of the tidal cycle would yield a variety of periods depending on the number of days used in the calculation and which days of the lunar month were used. Periods as short as 24.0 hours would be found for as much as 5 or 6 days out of any semi-monthly period. If other days were used, one might obtain periods as long as 25.8 hours. If, however, an entire semi-monthly period or a lunar month is used, the estimated period would be 24.8 hours. "Short" or "long" periods are restricted to particular times in a lunar period and thus are also limited to certain parts of the day. It is therefore evident that the apparent period can be described as a function of the phase relations between diurnal and tidal rhythms. It is obvious that period estimates obtained from peak-to-peak measurements are characteristic only for the days used for measurement.

It has been suggested by Barnwell (1963) that the alterations in rate of peak progression represent a simple additive effect. He has calculated mean diurnal and mean lunar-day curves for activity, and by recombining them in the appropriate phase relations has obtained composite curves closely simulating the actual recorded values. If such an effect provides much of the explanation for the apparent changes in period it is not clear why the effect is reduced when recording is done under conditions of natural illumination. As was seen in Figure 2B this situation provided a nearly constant period in that the relation of peaks to time of low tide showed no patterned variations but seemed to vary randomly around 2.5 to 3 hours before the time of low tide.

An alternative explanation of the apparent variations in peak progression is that the two cycles, diurnal and tidal, interact when in certain phase relations. The relative effectiveness of a driving force with a tidal frequency may be less when it is nearly in phase with a diurnal cycle. At such times the diurnal driving cycle entrains activity but when the tidal cycle reaches a certain phase angle difference with the diurnal one, the tidal cycle again assumes dominance over a portion of locomotor activity. There then occurs a transitional period when activity is reverting to entrainment by the tidal cycle, and periods longer than 24.8 hours are observed until re-entrainment in the original phase relations is complete. During this time the peaks of activity progress until they occur within an hour or less of time of low tide and thus reassume their previous phase relations.

Although there is no obvious reason why providing diurnal illumination should encourage full expression of the tidal rhythm it is, of course, possible that this is an effective factor. On the other hand, recording of activity under conditions of natural illumination began the day after new moon and ended just before full moon. Thus, there was increasing duration of moonlight during the recording period. It is possible that the additional light each night, while inadequate to shift diurnal rhythm, was sufficient to maintain the tidal rhythm. It could thus be regarded as a supplement to either an endogenous or an exogenous tidal cycle, its effect being to counteract the postulated temporary entraining power of the diurnal rhythm.

The major difference between the explanation here proposed and that suggested by Barnwell is that according to Barnwell any biological interaction would be at the level of locomotor activity *per se* and not between two rhythmical systems. He obviously assumes a constant period for the tidal rhythm, the expression of which is altered by association with peaks or troughs of a similarly stable diurnal rhythm. The changes in rate of peak progression would thus not represent true changes in period.

A second kind of relation between diurnal and tidal rhythms is seen in the consistency with which a shift in phase relations of the diurnal rhythm is accompanied by a shift of similar magnitude in the relations of time of activity peak to time of low tide. Such an induced shift persists in constant light and the tidal rhythm shows characteristics similar to the normal one when activity is recorded in constant illumination. It is not clear whether this situation indicates a coupling of some kind or simply similar sensitivities and responses.

The results recorded in Figure 4 for animals exposed to a 24.75-hour cycle can be interpreted as further illustration of the similarity of response or of linkage between diurnal and tidal rhythms in response to phase-shifting stimuli. Here the artificial period was produced by supplementing the constant low illumination with four hours of light, with the supplemental light period occurring 45 minutes later each day. On the first day of recording the supplemental light began at 10 PM and throughout the entire 11 days always began during the night. It has been shown that the diurnal rhythm of chromatophores is not shifted by one hour exposure to light but is shifted by six hours beginning during the night (Brown and Webb, 1949). The simplest explanation for the apparent period of about 25.5 hours seen in Figure 4A is that the four hours of light shifted the diurnal rhythm by about 45 minutes each time it came on and simultaneously shifted the tidal rhythm by about the same amount. There would thus be added to a 24.8-hour cycle the amount of the shift, yielding a period of about 25.5 hours. There is no other obvious way in which a 24.8-hour cycle imposed from without upon a natural 24.8-hour cycle could produce a period consistently longer than either. It should be noted that these experimental conditions, which might reasonably be expected to produce daily phase shifts in the activity cycle, are the only ones in which the activity peaks progressed for so long a time at a rate consistently different from the rate of tidal progression. In this feature they resemble the numerous so-called free-running periods reported in the literature for various circadian rhythms. The possibility has been suggested (Brown, 1959) that these free-running periods observed under constant conditions may be the result of daily phase shifts produced because the sensitivity fluctuates throughout a 24-hour period.